Advanced Journal of Microbiology Research Vol. 2005
Available online at http://internationalscholarsjournals.org/journal/ajmr

Full Length Research Paper

Production and partial characterization of a thermostable amylase from ascomycetes yeast strain isolated from starchy soils

Bertrand Tatsinkou Fossi[1], Frédéric Tavea[1], Robert Ndjouenkeu[1*]

Department of Food Science and Nutrition, University of Ngaoundéré, P.O.Box 455 Ngaoundéré, Cameroon.

Accepted 14 july, 2004

An amylolytic yeast strain was isolated from starchy soils (flour mills environment, flour market, cassava farms after harvesting and transformation of tubers) and its enzyme productivity and activity evaluated. The enzyme synthesis was optimum at 30°C when initial pH of fermentation medium was 4.5. After extraction and partial purification, the enzyme remained stable in a range of temperature and pH between 20°C-60°C and 2-8 pH respectively. The optimum enzyme activity was displayed at 70°C and pH 5.5 and 6.5. The thermostability of the enzyme was comparable to that of amylases from bacteria.

Key word: Amylolytic yeast, amylase activity, thermostable amylase.

INTRODUCTION

Amylases are among the most important enzymes used in biotechnology, particularly in process involving starch hydrolysis. Though amylases originate from different sources (plants, animals and micro organisms), the microbial amylases are the most produced and used in industry, due to their productivity and thermostability (Burhan et al., 2003). Natural fermented media (foods and soils) offer the substrates for isolation of micro organism strains producing amylases. In this respect, many strains used in food industry originate from fermented food media, while soils, particularly wastes and mud offer strains used mainly in chemical industry (Ramesh and Lonsane, 1989; Burhan et al., 2003; Gomes et al., 2003).

Soils around mills, cassava farms after harvesting and treatment of tubers, and flour markets represent media where natural amylolytic and fermented activities occur. Due to the fact that starch constitutes the main substrate of these media, amylolytic microorganisms are thus supposed to be naturally present there.

The present work is aimed at investigating these media, in order to isolate yeast strains producing amylolytic enzymes, and to characterise the strain productivity and the enzyme produced, particularly their behaviour toward temperature and pH. The choice of yeast isolation rather than bacteria was justified by the facility of their culture and their harmlessness.

MATERIAL AND METHODS

Source and preparation of soil samples

Soil samples were collected from starchy wastes of cassava plantations, flour markets and mills, using aseptic bags.

*Corresponding author. E-mail: rndjouenkeu@yahoo.fr.

Isolation and partial identification of yeast strain

The yeast strains were isolated from soil samples according to the method of Min et al. (1999). The soil sample was at first enriched on a liquid medium made of 1% soluble starch, 0.5% peptone (w/v), 0.5% yeast extract (w/v), at pH 3.0. 1g of soil was mixed with the medium placed in tubes, and incubated at 25 to 28°C for 3 days with shaking. 0.2% of the enriched liquid was then sowed on solid medium (1% soluble starch, 0.5% peptone, 1.5% yeast strain, 2% agar, 0.5 g/ml chloramphenicol), using petri dishes (Marchal, 1976; Min et al., 1999). The petri dishes were incubated at 25 to 28°C for 48 to 72 h until yeast typical colonies were obtained. The colonies were purified several times on agar plate. Morphological characteristics of isolates were identified according to the yeast taxonomy (Kreger, 1984).

Amylolytic activity of yeast strains

The amylolytic activity of yeast strains was determined by introducing an equal number of cells of each yeast isolate in a 2 mm hole, aseptically made in the agar medium. The agar plate was then incubated for 48 h at 30°C, after which a 4% lugol solution was added to the medium. The diameter of halo formed after addition of lugol was measured and represented the amylolytic activity of the strain. The most active strain was used for former characterisation.

Enzyme productivity of selected yeast strain

Selected yeast strain was propagated at 30°C for 48 h, in 50 ml of a 8% starch medium placed in 250 ml flask, under shaking. The effect of carbohydrate source on the productivity of the strain was studied by running the propagation on different starch media: commercial soluble starch, corn, cassava, rice, wheat and potato. In addition, the propagation was undergone at different starch concentrations (1 to 15%, w/v), different temperatures (20 to 55°C) and different pH (5 to 8) using the most productive fermented medium. The initial pH of the medium was adjusted using sodium hydroxide and sulphuric acid.

At the end of each fermentation batch, yeast cells were counted on a portion of fermented broth using Thomas cell, while the remaining broth was centrifuged at 8000 rpm for 15 min., and the supernatant collected as crude enzyme extract. A portion of the crude extract was used to determine the enzyme productivity of the strain through measurement enzyme activity, while the remaining extract served for partial purification and characterisation of amylase.

Partial purification of amylase

A solution of 65% (w/v) sodium sulphate was added to the clear supernatant and enzyme recuperated by centrifugation at 8000 rpm. The enzyme precipitate was then suspended in phosphate buffer 0.005M, pH 6.0. The purified extract was used to determine enzyme activity and properties.

Enzyme assay

Amylase activity both in crude and partially purified extracts was assayed using soluble starch as substrate, according to the method of Keleke et al. (1998). The amylase activity was defined as the amount of soluble starch hydrolysed by 1 ml of enzyme extract in 60 min. The optimal temperature for activity was determined by assaying activity between 20 and 100°C. Thermostability of the amylase was performed by maintaining the enzyme solution in water bath at different temperature (20 to 100°C) for 30 min, then cooling and running the activity assay. Measurement of optimum pH for amylase activity was carried out by running the activity assay between the pH range of 2.0 to 10.5, using 0.05 M Na_2HPO_4-citrate, tris-HCl and glycine-NaOH as buffer solutions. The pH stability was determined by incubating partially purified enzyme in water bath at 70°C and measuring the residual activity. All measurements were undergone at least in triplicate.

RESULTS AND DISCUSSION

Identification and amylolytic activity of yeast isolates

Twelve yeast strains presented significant amylolytic activity (Figure 1). Since the distinction between the strains laid only on the soil origin and taxonomy characteristics of isolates, it was not possible to define whether amylolytic variation between the strains was the consequence of species variability or environment effect on the same micro organism. Thus, the strain BA_3 showing the highest amylolytic halo was selected for further analysis. The propagation of the selected strain BA_3 on different starch media showed higher amylase production and activity on wheat (Table 1).

Table 1. Effect of starch source on amylase production.

Starch source (8% w/v)	Apparent Amylase activity (g/ml/h)
Wheat	298,5±0,1
Corn	200,0±2,8
Potato	103,5±3,2
Rice	97,3±1,7
Soluble starch	80,0±0,3
Cassava	78,0±1,2

Enzyme productivity of the yeast strain

The measurement of biomass an amylase activity in 8% wheat fermented broth during 48 h showed that amylase production increased with cell growth; the maximum amylase being recovered after 45 min of fermentation (Figure 2). In addition, the enzyme production was optimum at 30°C and pH 4.5 (Figure 3) . Kocher and Katyal (2003) found comparable trend in amylase productivity of *Shwanniomyces occidentalis*, a yeast strain isolated from potato fermentation.

Thermo stability and ph stability of the amylase

The enzyme remained stable at a temperature range between 20 and 60°C, above which the stability a rapidly declined. The maximum activity was displayed at 70°C

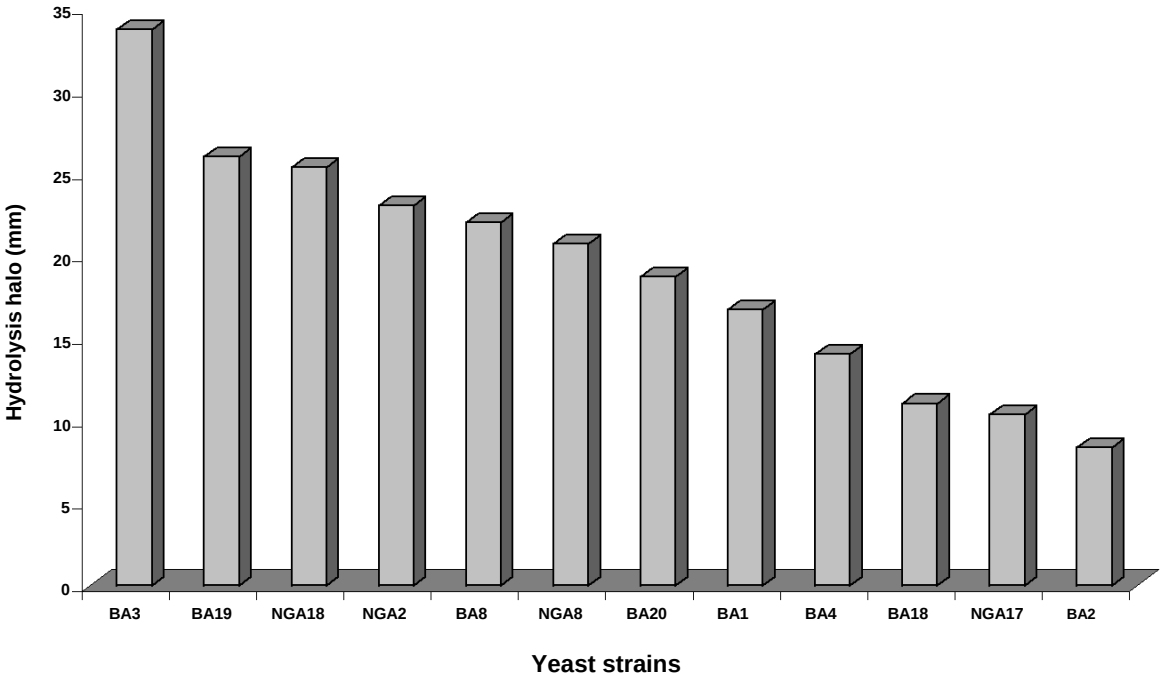

Figure 1. Amylolytic activity of isolated yeast strains after 48 h fermentation at 30°C.

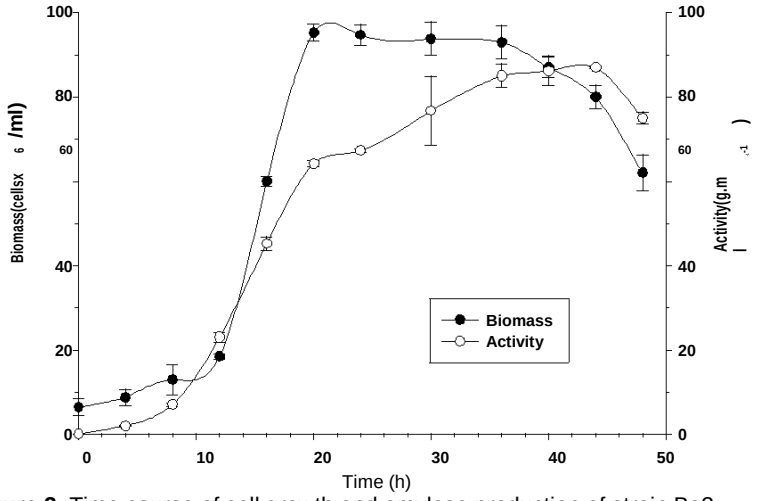

Figure 2. Time course of cell growth and amylase production of strain Ba3.

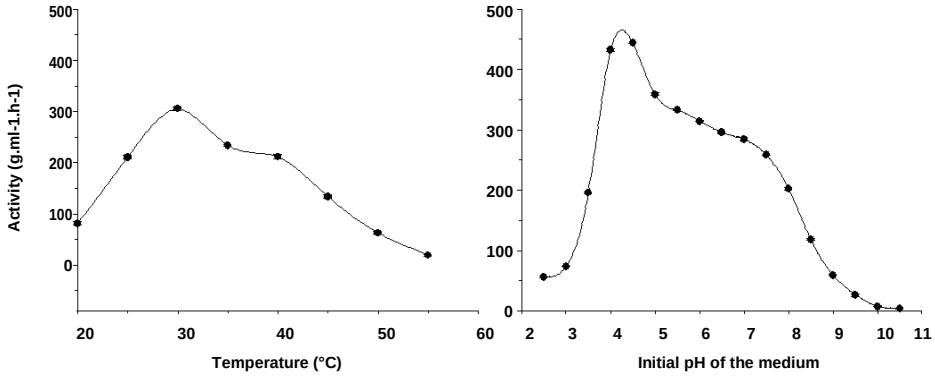

Figure 3. Effect of temperature and initial pH of the medium on amylase production.

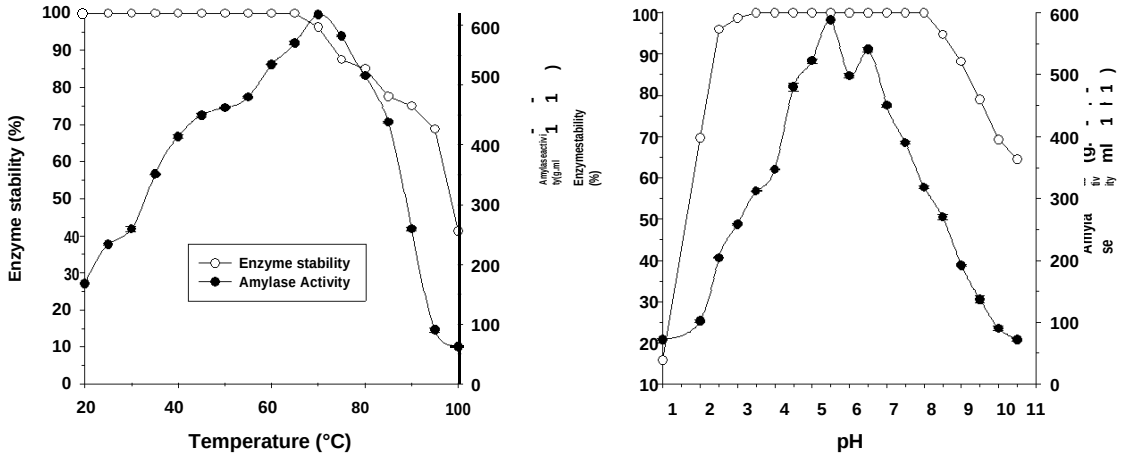

Figure 4. Effect of temperature and pH on the enzyme activity and stability.

(Figure 4). The above enzyme stability trend is comparable to the behaviour of amylase from some bacteria genus such as *Bacillus licheniformis* (Morgan and Priest, 1981) and does not fit the general behaviour of fungi and yeast amylases which naturally are not thermo stable (Barbier, 1997), except if they have been genetically modified. Amylase of the strain BA_3 appears thus as an exception. In addition, the optimum activity temperature of strain BA_3 amylase is higher than that of some known fungus amylases such as *Aspergillus niger, Aspergillus orizea, Mucor pusillus* (Fogarty, 1983), but remains comparable to amylases from *Bacillus lentus* (El-Aassar et al., 1992), *Bacillus coagulans* (Fatma and El-Refai, 1991) and *Bacillus caldolyticus* (Heinein and Heinein, 1972) whose optimal activity is equally displayed at 70°C.

The effect of pH on amylase activity was characterised by stability from pH 3.0 to pH 8.0 after 30 minutes of incubation at 30°C. The optimum pH activities were 5.5 and 6.5 (fig 4). This may indicates a poly enzyme structure of the BA_3 amylase.

The above behaviour of the BA_3 amylase, particularly their thermo stability, brings out some hypothetic considerations, taking into account the natural medium where the yeast strain have been isolated. Bacterial fermentation is the main phenomenon taking place in starchy soils of flour mills. The bacteria considered are usually thermo-resistant. Cohabitation of bacteria and yeast in such a medium may have induced gene transfer from bacteria to yeast, justifying the unusual thermal behaviour of the yeast strain BA_3 amylase, comparable to amylase from bacteria. Such genetic transformation occurs when different micro organisms are cultured on the same medium (Gasson et al., 1984; Hofer, 1985; Deak et al., 1986; Cocconcelli et al., 1986). Composition of the medium and environmental conditions are among factors governing the process. The above hypothesis suggests then scientific interest in investigating bacteria amylase from flour mill soils and in comparing it with yeast amylase from the same medium.

REFERENCES

Barbier G (1997). Les enzymes des micro-organismes « extrêmophiles » : une nouvelle source en cours d'exploration. In : Larreta-Garde V. (ed.) Les enzymes en agroalimentaire, Tec et Doc Lavoisier, Paris, pp. 308-347

Burhan A, Nisa U, Gökhan C, Ömer C, Ashabil A, Osman G (2003). Enzymatic properties of a novel thermostable, thermophilic, alkaline and chelator resistant amylase from an alkaliphilic *Bacillus* Sp. isolate ANT-6. Process Biochem. 38: 1397-1403.

Cocconcelli PS, Morelli L, Vescovo M, Bottazzi V (1986). Intergeneric protoplast fusion in lactic acid bacteria. FEMS Microbiol. Lett. 35(2-3): 211-214.

Deak M, Kiss GB, Koncz C, Dudits D (1986). Transformation of Medicago by Agrobacterium mediated gene transfer. Plant Cell Rep. 5(2): 97-100.

El-Aassar SA, Omar SH, Gouda MK, Isah AM, Abbdel-Fattah AF (1992). Purification of α-amylase from *Bacillus lentus* cultures. Appl. Microbiol. Biotechnol. 38: 312-314.

Fatma JE, El–Refai A (1991). Purification ad characterization of α-amylase by a thermophilic isolate of *Bacillus coagulans*. Chem. Mirobiol. Technol. Lenbensm. 13:102-110.

Fogarty W (1983). Microbial enzymes and biotechnology. Applied Science publishers, London and New York.

Gasson MJ, Davies FL (1984). The genetics of dairy lactic-acid bacteria. In: Davies FL and Law BA (eds). Advances in the microbiology and biochemistry of cheese and fermented-milk. Elsevier Applied Science Publishers Ltd.; Barking, Essex; UK, pp. 99-126.

Gomes I, Gomes J, Steiner W (2003). Highly thermostable amylase and pullulanase of extreme thermophilic eubacterium *Rhodothermus marinus*: Production and partial characterization. Bioresour. Technol. 90: 207-214.

Heinein UJ, Heinein W (1972). Characterization and properties of a caldo-active bacterium producing extracellular enzymes and two related strains. .Arch. Microbiol. 82: 1-13.

Hofer F (1985). Transfer of lactose fermenting ability in *Lactobacillus lactis*. New Zealand J. Dairy Sci. Technol. 20(3): 179-183.

Keleke S, Agbor E, Brauman A, Griffon D (1998). Origine de l'activité amylasique dans la fermentation lactique des racines du manioc. In Agbor E *et al.*(eds) Transformation alimentaire du manioc. ORSTOM, Paris, pp. 319-328.

Kreger Van Rij (1999). The yeast: a taxonomy study, 3rd Ed. Elsevier, Amsterdam.

Kocher GS, Katyal P (2003). Use of potato starch for extracellular amylase production by a yeast isolate. Department of microbiology, Punjab agricultural university, Ludhana 141004, India. www.cassa.org/News/Biothailand

Marchal (1976). Initiation à la microbiologie. Dunod, Paris.

Min H, Zhao Y, Zheng P, Wu W, Cheng S (1999). Microbial research technique. Science Press, Beijing.

Morgan FJ, Priest FG (1981). Characterization of a thermostable α-amylase from *Bacillus licheniformis* NCIB 6346. J. Appl. Bacteriol. 50: 107-114.

Ramesh MV, Lonsane BK (1989). Solid state fermentation for production of higher titres of thermostable alpha-amylase with two peaks for pH optima by *Bacillus licheniformis* M27. Biotechnol. Lett. 11(1): 49-52.